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TECHNICAL SUPPLEMENT

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ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

Under the above caption will be published from time to time comments, criticisms and suggestions on technical procedures; minor contributions such as laboratory aids and short cuts which are not considered sufficiently important to warrant a formal paper; and queries.

Obviously comments and criticisms should be signed; queries should be signed but names will be withheld on request; full credit will be given those who contribute laboratory aids, short cuts and the like.

An attempt will be made to obtain answers from authoritative sources to the queries submitted. It must be emphasized that the views expressed in this department are not the opinions of any official body.

THE USE OF DIOXAN AND ACETONE FOR THE RAPID DEHYDRATION AND PARAFFIN EMBEDDING OF FIXED TISSUES*

I. A. NELSON

From the Pathological Laboratories of St. John's Hospital, Tulsa, Oklahoma, aided by the Mark Finston Research Fund

In the fall of 1934 Dr. S. B. Pessin, Pathologist for St. Mary's Hospital at Madison, Wisconsin, sent me a mimeographed copy of all the literature which Dr. H. W. Mossman of the University of Wisconsin had collected on the use of dioxan up to November 1933. This mimeographed copy contained Dr. Mossman's own experiences and translations of two articles which he found in the German literature[1,2]. Since 1933 there have been many articles on the use of dioxan [3, 5, 6, 7] which bring out the following features:

1. It dehydrates fixed tissues with little shrinkage.
2. It does not harden tissues as much as xylol, alcohol, and chloroform.
3. It can be used in combinations as a rapid fixative.
4. It is miscible with many substances such as water, alcohol, formaldehyde, acetic acid, acetone, and even melted paraffin.
5. Calcium chloride lumps are insoluble and when kept in the dioxan remove water and alcohol sufficiently to eliminate the necessity of passing tissues into several solutions.
6. A fair degree of volatility permits its use in the place of xylol or chloroform when passing into paraffin.
7. It can be used for a sufficient number of tissue blocks to result in a diminished net cost. It becomes quite dark with lipoids and tissue extractives before it needs to be changed.

* Methods and apparatus shown at the Scientific Exhibit Section of the Fifteenth Annual Meeting of the American Society of Clinical Pathologists, May 6 to 10, 1936, Kansas City, Missouri.

8. It can be used to cut down the time and work in modified paraffin and freezing methods.

9. It can be easily removed from sections and thus not interfere with staining.

10. It can produce severe toxic changes to the liver and kidneys of workers exposed to concentrations of 1 to 1000. Also, its action is cumulative, but by using well sealed containers, exposure can be reduced to a minimum.

The properties brought out in the earliest publications led to experiments from which the rapid paraffin embedding method of McNamarra[8] was modified as follows:

1. Fix tissues with formalin or Bouin's solutions.
2. Dehydrate tissue blocks for 30 to 90 minutes with dioxan. The blocks are supported above the calcium chloride kept in the dioxan.
3. Transfer to acetone for about the same time. Calcium chloride lumps are kept also in the acetone.
4. Transfer blocks directly into 56°C. melted paraffin for an hour or longer.
5. Block tissues directly with the same paraffin used in step 4.

The above outline covers literally all there is to this modified method which has been used routinely for two years.

The following accessories have been developed for use with this method and are described under separate titles:

1. Wide mouth dehydrating jars with mercury seal for ease of manipulation, control of evaporation, and to prevent moisture absorption from the air.

2. Non-metallic and non-fragile porous cups and baskets. The cups are used for isolation and transfer of tissue blocks from dioxan to acetone. The baskets hold the cups above the calcium chloride.

DISCUSSION

Imperfectly fixed tissues do not make good sections. It is well to repeat that "formaldehyde is generally regarded as a rapid penetrator but as taking a long time to exert its full effects"[3]. Also, delayed fixation may alter the number and character of mitotic figures in a few minutes, thus clouding the microscopic interpretation[4]. It is true that blocks of tissue can even be

boiled in formalin solutions for rapid fixation but we prefer to leave tissues in 10 per cent formalin or Bouin's overnight.

No attempt has been made to determine quantitatively the lipoid extraction but cholesterols and neutral fats appear to come out of tissues readily.

Acetone extracts the dioxan, some lipoids, and traces of water rapidly. It does not seem to make tissues as brittle as xylol or chloroform. It enhances the usefulness of dioxan for rapid dehydration. These are important characteristics and have much to do with the utility of this method for routine hospital surgical tissues. McNamarra stresses in his paper that "The secret of success in this (his) method lies: (*a*) In the avoidance of any of the commonly used clearing agents (xylol, chloroform, toluol, benzol, etc.)"[8]. When the tissue is dropped into melted paraffin the acetone rapidly evaporates leaving a dry tissue into which the paraffin permeates directly. A word of caution is needed at this point. No impregnating fluids will penetrate areas which contain trapped gases. Since acetone is not soluble in paraffin the blocks should be turned over if they contain cystic areas which are of such a nature as to effectively trap acetone in the gaseous state. The routine use of acetone after dioxan for two years has shown that this rarely occurs. Another criticism which may be made of this method is that occasionally sections tend to drop out of a ribbon. This is not understood. However it has not been sufficiently troublesome to make us want to go back to any of the older methods. It apparently may occur with dioxan alone or with acetone after dioxan.

The 56°C. melted paraffin is not thought to be too hot[3]. Since this temperature is near the boiling point of acetone it speeds up the rate of impregnation. Furthermore we have found that this grade of paraffin can be used in the hottest weather by supporting a piece of dry ice in a small wire-mesh basket directly over the microtome blade and paraffin block. The dry ice is covered over and allowed to thaw mostly from the under surface. The heavy cold vapors settle down over the parts to be chilled.

Paper cups can be kept filled with melted paraffin in the compartments of aluminum trays such as are used in modern electric

refrigerators. When the blocks have been in the melted paraffin a sufficient length of time the cups can be transferred directly into cool water.

Thus the use of acetone assures rapid dehydration and eliminates the necessity of two or more paraffin steps. Since we use acetone after dioxan, we believe that most of the dehydration has already taken place and therefore this final rapid dehydration in acetone produces little added shrinkage.

The question of shrinkage always comes up with any method of rapid tissue technique. Some writers who use dioxan throughout their dehydration and embedding claim that dioxan shrinks tissues less than the usual reagents [3, 5, 6]. However we do see shrinkage in some surgical tissues. There seem to be at least five causes of shrinkage which may be listed as follows:

1. All methods of rapid dehydration increase the chances for shrinkage.

2. Some tissues call for special methods. Areolar tissues shrink badly. Not all tissues with high lipoid content shrink. Brain and some ovarian tumor tissues go through this rapid dehydration quite satisfactorily. All parenchymatous structures stand up remarkably well even when parts of connective tissue elements suffer some shrinkage.

3. Delayed fixation and incomplete fixation naturally cause changes at times but dioxan and acetone do not exaggerate these faults as much as the usual slow methods.

4. Pathological changes in the tissue may cause affected areas to decrease their resistance to rapid dehydration. These are not so hard to recognize and interpret.

5. Sections may become shrunken after cutting and in the staining process. Some workers use dioxan for deparaffining their sections[1, 5].

When due allowance is made for the last four causes of shrinkage, we feel that what shrinkage occurs as a result of rapid dehydration is of minor significance and has not interfered with the study of routine sections.

The time gained for sectioning and staining has made possible routine reports within twenty-four hours without resorting to

decreasing the size of the blocks nor rushing through fixation. Small bits of tissue can be pushed through in three hours by compromising on rapid fixation.

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WIDE MOUTH DEHYDRATING JAR WITH MERCURY SEAL*

I. A. NELSON

From the Pathological Laboratories of St. John's Hospital, Tulsa, Oklahoma, aided by the Mark Finston Research Fund

The following is a description of a method for making a mercury seal on the ordinary wide mouth hospital "dressing jar" used for storing sterilized gauze dressings, bandages, etc. It was developed with the following criteria in mind: economy, simple construction, wide mouth, ease of operation, and a dependable control of evaporation and moisture absorption.

* Methods and apparatus shown at the Scientific Exhibit Section of the Fifteenth Annual Meeting of the American Society of Clinical Pathologists, May 6 to 10, 1936, Kansas City, Missouri.

MATERIALS

1. Dressing jar with glass cover. We use the size measuring 4 by 4 inches. The Pyrex forms do not have a cover which overlaps the top as far down the side as other makes so they are not recommended.

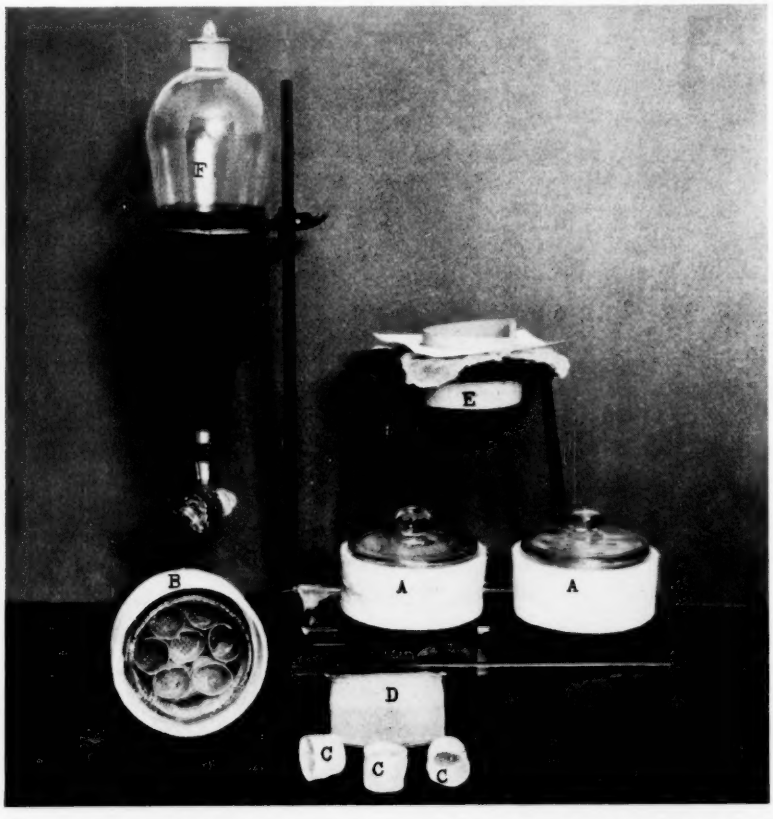

FIG. 1. *A*, dehydrating jars in routine use; *B*, dehydrating jar on side to show basket and cups; *C*, porous cups made with fillet net and casein glue; *D*, basket to hold cups in dehydrating jars; *E*, basket parts assembled to suggest construction; *F*, separatory funnel for recovering solutions.

2. Rubber bands. Inner tubes not over 4 inches in diameter or several cuffs from rubber gloves can be cut so as to make bands about 1½ inches wide.

3. A roll of 4-inch plaster of Paris bandage.

PROCEDURE

1. Slip the rubber band over the glass cover so that one edge of the band coincides with the edge of the cover. Place the cover on the jar.

2. Soak the roll of plaster of Paris bandage and wrap several layers quickly around the jar and cover in such a manner that the cover is efficiently sealed in position with a collar about ¼-inch thick. Technicians in hospitals should get

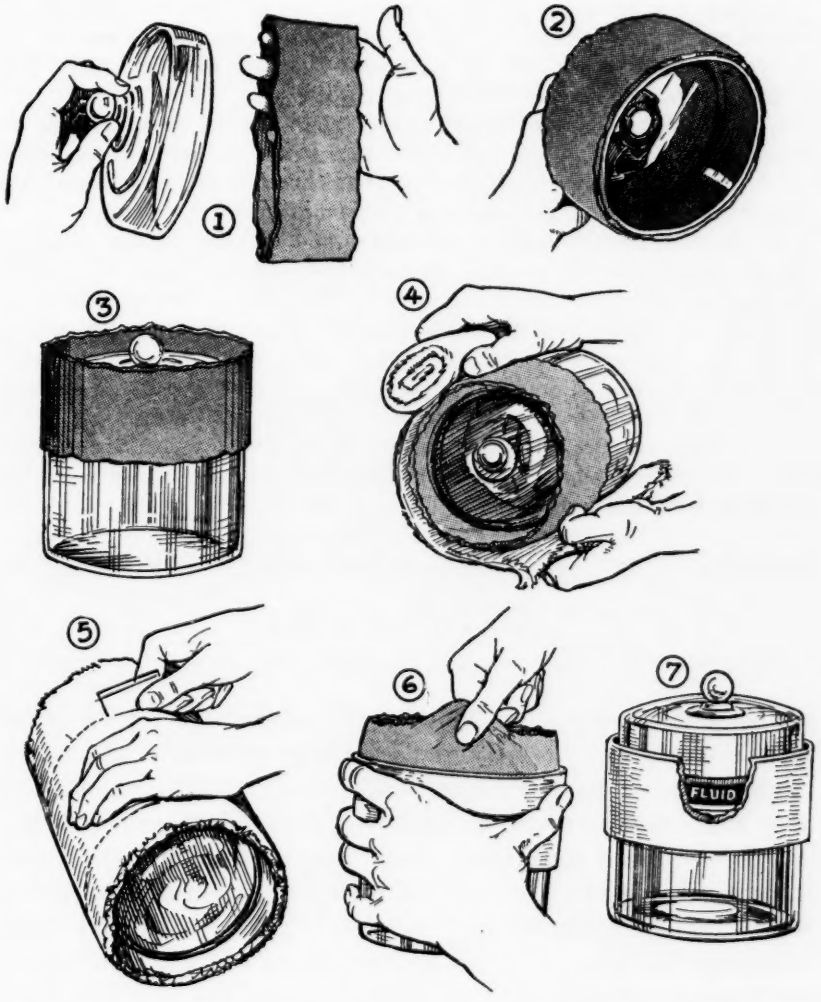

FIG. 2. SHOWING STEPS IN THE PREPARATION OF THE DEHYDRATING JAR

the help of some one in the orthopedic department. Other technicians should experiment with plaster of Paris as it sets quickly and it is a little difficult to make a smooth job the first time.

3. Trim both edges before the plaster of Paris sets too firmly. This can be

done easily by rotating the jar while cutting the plaster of Paris bandage with a razor blade. Try to avoid cutting entirely through the rubber band when trimming the upper edge. The trimmed edges can be smoothed so that no loose threads are exposed. The trimmed collar should be about 2 inches wide.

4. When the plaster of Paris has set pull the rubber out. Rubber was selected particularly because it can be teased off. As soon as the rubber slips out the cover can be lifted off and replaced in the moat with ease. The glass surfaces can now be cleaned with a moist cloth.

5. Pour about a quarter of a pound of mercury into the space between the plaster of Paris collar and the top of the jar. It will be found that the cover now floats on the mercury. If the proper thickness of rubber was used, the cover will have free play and float on a minimum quantity of mercury.

We use these jars for dehydrating tissues with dioxan and acetone. Calcium chloride lumps keep the reagents practically anhydrous. When the calcium chloride has absorbed so much water that it is nearly reduced to a brine, we take the cover off and pour the entire contents into a separatory funnel or large funnel with a rubber tube and pinch clamp on the spout. The mercury goes to the bottom and can be drawn off immediately. The brine soon forms a layer which can be discarded. The acetone or dioxan can now be recovered.

Since the covers are occasionally drawn off a little hurriedly some mercury is sprayed out of the moat. When this falls outside it may be lost unless the jars are kept in some sort of tray.

IMPROVED METHOD FOR RAPID DECALCIFICATION*

ELENA DE GALANTHA

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The difficulties which confront the laboratory worker when there is a demand for cutting and staining sections of tissue containing bone or lime salts are well known. If the calcified or bony mass is very dense or large, the decalcification procedures usually require a long time (sometimes weeks), and the corresponding damage by the chemical used is apparent in the staining of the cellular elements and other soft parts. Cell and tissue differentiation often is nearly or completely absent. Because of the necessary prolongation of the process and the harmful effects to elective staining reactions, microscopic study of such tissues is not only unsatisfactory but often is not undertaken at all.

* Submitted for publication January 14, 1937.

This is particularly true when such structures as compact bone, the petrous portion of the temporal bone, teeth, or the masses found in calcified aortic stenosis, are to be examined microscopically.

Any method, therefore, which would hasten the decalcification and at the same time preserve the staining properties of the tissue would be a desirable addition to the armamentarium of a technical laboratory. After a considerable period of experimentation, the following formula was evolved and is suggested as a method for improving the technic as generally employed.

1. The bony or calcified tissue should be fixed in 10 per cent formalin for at least 24 hours.

2. Blocks for sectioning should be cut to appropriate dimensions (the smaller and thinner, the better) and placed in the following mixture:

(a) Add 20 cc. of concentrated nitric acid to 50 cc. of distilled water.

(b) Add this solution (*a*) slowly to 20 cc. of 95 per cent ethyl alcohol. (This should be done cautiously, using cold solutions.)

(c) Add 10 cc. of concentrated picric acid.

(d) Add 10 cc. of pure olive oil and mix thoroughly.

3. At the end of 24 hours in this acid mixture, test the extent of decalcification by a fine needle or knife, and thereafter at shorter intervals until the consistency is that of soft tissue.

4. Wash thoroughly in running tap water for 12 hours.

5. Place blocks in 2 per cent ammonia solution for 24 hours.

6. Wash again for 12 hours in running water.

7. Dehydrate, embed in paraffin, cut, and stain in the usual manner.

The oil apparently prevents excessive hardening and dehydration of the calcareous tissue during the process of decalcification. At least the lime salts in this solution are more rapidly removed than by the usual acid mixtures and the staining properties of the tissue more perfectly preserved.

PREVENTION OF ADVENTITIOUS PRECIPITATE FORMATION IN FONTANA STAIN PREPARATIONS

B. S. KLINE

The following Fontana method has been found invariably satisfactory for the silver impregnation of spirochetes:

Make smear in usual manner.

Allow to dry in air.

Place in fixative in Coplin jar one to two minutes. (Fixative: 100 cc. distilled water; 1 cc. acetic acid; 2 cc. concentrated formalin.)

Wash in running water about 1 minute.

Blot.

Cover with mordant (5 per cent tannic acid in 1 per cent phenol).

Steam about 1 to 2 minutes.

*Wash under a vigorous jet of tap water for several minutes until ALL excess mordant removed.**

Blot.

Partially cover slide (including all of smeared portion) with 2 per cent silver nitrate solution.

Allow a few drops of dilute ammonia to mix with the silver nitrate. (Dilute ammonia: 1 cc. of concentrated household ammonia to 60 cc. of distilled water.)

Steam about 1 to 2 minutes.

*Float off stain with tap water. Prevent surface metallic looking material from touching smear. Wash well with tap water.**

Blot.

Warm until dry before examination.

The tannic acid mordant is delivered from a glass stoppered 2-ounce dropping bottle.

The 2 per cent silver nitrate solution is delivered from a 2-ounce brown dropping bottle (glass stoppered).

The dilute ammonia is delivered from a glass stoppered 2-ounce dropping bottle.

* If these two steps are carried out properly, no adventitious precipitate will be found on the slide.

All solutions keep well for months.

The fixative solution and the tannic acid in phenol solution are replenished from larger stock bottles; the silver nitrate solution and dilute ammonia are made once every several months to refill the dropping bottles.

SIMPLE TESTS FOR MERCURY IN BODY FLUIDS AND TISSUES*

ALEXANDER O. GETTLER

From the Chemical Laboratories of Bellevue Hospital and of Washington Square College, New York University, New York City

The tests to be described are modifications and further developments of the Reinsch Volatilization test.

Five to 10 grams of hashed tissue, stomach contents or concentrated urine are placed into a 25 cc. test tube, and 3 cc. of concentrated HCl are added. The tube and contents are placed into a boiling water bath. A clean bright copper wire spiral (wire 20 gage) is introduced into the test tube and the tube and contents are allowed to remain in a boiling water bath for 30 minutes. Remove the spiral, wash it with water, alcohol, and finally ether. A dark deposit may be due to arsenic, antimony, bismuth, silver or mercury. The deposit due to mercury is usually greyish or silvery. If only traces of any of these metals were present, the deposit may not be definitely visible. In either case proceed further. Mercury can be quickly spotted by the following simple tests:

1. Place a small filter paper on a watch glass; then place one drop of a suspension of cuprous iodide† upon the filter paper. As the water in the drop is

* Read before The Fifteenth Annual Convention of The American Society of Clinical Pathologists at Kansas City, Missouri, May 8 to 10, 1936. Received for publication, July 24, 1936.

† The cuprous iodide is made according to Ganassini. Five grams $CuSO_4$ + 3 grams $FeSO_4$ (anhydrous, precipitated with alcohol) are dissolved in 100 cc. of water. Seven grams KI in 50 cc. H_2O are added while stirring. The precipitate is filtered and washed with water. The precipitated cuprous iodide is then transferred to a brown glass bottle with the aid of a little water. It is kept in the form of a suspension.

absorbed by the filter paper, it leaves an almost colorless spot of cuprous iodide. Now cut off the stem of the copper spiral containing the deposit, and place the spiral directly on the spot of cuprous iodide, and cover with another watch glass. Let stand about ten to fifteen minutes. If even a trace of mercury is present, a rose to salmon color develops, due to the formation of mercury iodide.

2. To prove further the presence of mercury, another copper wire spiral deposit is obtained as above described. The spiral after being washed with water, alcohol and finally ether, is placed into a small volatilization tube (2.5 cm. long, 0.75 cm. diameter). The latter is then adjusted through a snug hole in a thick sheet of asbestos (*D*), as indicated in the diagram. A microscopic slide (*A*) is placed over the mouth of the volatilization tube. A piece of ice is placed upon the slide to keep undersurface cold. By means of a micro burner (*F*) the lower part of the volatilization tube is heated to about 300°C. for about one minute. Care must be taken not to allow part of the tube above the asbestos

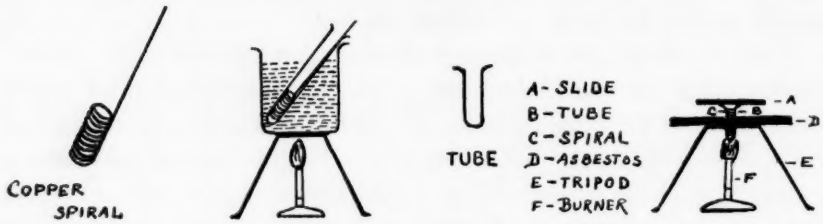

FIG. 1

sheet to get very hot, and the microscopic slide must remain cold. During the heating any mercury present on the spiral will volatilize and deposit on the cool slide as metallic mercury; on examining this deposit under the microscope, perfectly round, tiny, silvery droplets are seen. If viewed by reflected light only, each globule can be seen glistening. The deposit on the slide can be further identified as mercury in the following manner: Place a crystal of iodine upon the slide, very close to the mercury deposit. Cover both the mercury deposit and the crystal of iodine with a small watch glass. Put the slide in a slanting position (angle of 30° with table) in a warm part of the room. The sublimed iodine vapors being quite dense will run down the inclined slide, come in contact with the mercury globules and form orange or red mercuric iodide. Microscopic examination of the specimen now will reveal orange red rhombic and tetrahedral crystals.

The tests described have been used for many years in our laboratories. They have been found to be rapid, sensitive and specific for mercury.

A RAPID AND SENSITIVE TEST FOR FLUORIDE IN BODY FLUIDS AND TISSUES*

ALEXANDER O. GETTLER AND LESTER ELLERBROOK

In a toxicological examination of a suspected fluoride poisoning it is essential to prove the presence of fluoride not only in stomach contents but also in the other internal organs.

The quantity of fluoride in the organs of a fatal fluoride case is of the order of 0.05 mgm. in 5 grams of tissue.

The classical etch test is not sensitive enough to detect the fluoride even if one uses as much as 100 grams of tissue; 500 grams or more must be used. Then again great care is needed to prevent loss of fluoride during ashing. This process makes the procedure very tedious and time consuming.

We recommend the following method because of its sensitivity and rapidity.

1. Five grams of hashed tissue are treated with 5 cc. of water and 2 cc. concentrated nitric acid and thoroughly mixed. After fifteen minutes 15 cc. of water are added with constant stirring.

2. The solution is then filtered through a Büchner filter with suction.

3. To the filtrate is added 0.5 gram solid lathanum acetate and then solid ammonium acetate until alkaline to methyl orange. A gelatinous precipitate appears.

4. The suspension is then boiled a few minutes to coagulate the precipitate and then filtered through a Gooch crucible with an asbestos mat.

5. The mat and precipitate are placed into a porcelain crucible, and dried on a hot plate.

6. To the dried material a little powdered glass is added and mixed.

7. A few drops of concentrated H_2SO_4 are then added, and the crucible immediately covered with a microscopic slide having a drop of water on its lower surface.

8. The crucible is then placed on a heating block at 150°C., and a small beaker containing ice water is placed on the slide to keep it cool.

9. After three to five minutes the slide is removed and a drop of saturated NaCl solution is added to the drop on its surface.

Crystals of sodium fluosilicate develop. They are hexagonal in shape and if

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* From laboratories of the Chief Medical Examiner's Office and of Washington Square College, New York University, New York City. Received for publication, July 24, 1936.

viewed with somewhat subdued light they have a faint pink color. Sensitivity, 0.01 mgm. in 5 grams of tissue.

Reactions

$$2LaF_3 + 3H_2SO_4 \rightarrow La_2(SO_4)_3 + 6HF$$
$$4HF + SiO_2 \rightarrow SiF_4 + 2H_2O$$
$$3SiF_4 + 4H_2O \rightarrow H_4SiO_4 + 2H_2SiF_6$$
$$H_2SiF_6 + 2NaCl \rightarrow Na_2SiF_6 + 2HCl$$

Traces of fluoride are normally present in human organs. The quantity present is of the order of 0.001 mgm. in 5 grams. If therefore, 50 grams or more of normal tissue are analyzed, two or three crystals may develop; but if only 20-gram portions are taken for analysis, no crystals form because the quantity of fluoride present in 20 grams of normal tissues is below the sensitivity of the test. In cases of fluoride poisoning even 10 grams or less of tissue gives many typical sodium fluosilicate crystals.

Stomach contents and urines from acute cases contain larger amounts of fluoride than do the organs. The method can therefore be simplified by using a smaller sample and omitting the removal of protein: One cubic centimeter (1 gram) or less, even one drop of stomach contents or concentrated urine is placed in a porcelain crucible. Sodium hydroxide solution is added until definitely alkaline. The material is dried on a hot plate, then mixed with a little powdered glass. From here on the procedure outlined above is followed.

The test described has been used in the toxicological laboratories of the Chief Medical Examiner's Office, of New York City, for the past two years, with excellent results. Four cases of fluoride poisoning that lived several days after taking the poison were detected by this test, whereas the etch test resulted negatively.

PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

DETERMINATION OF CHLORIDES IN BODY FLUIDS BY USE OF ADSORPTION INDICATOR

ABRAHAM SAIFER AND MORRIS KORNBLUM, J. Biol. Chem., **112:** 117–122. 1935

The proteins of the serum are precipitated by the use of a 3:1 alcohol-ether solution.

Solutions:

I. Standard sodium chloride.

Solution A:

10,000 mgm. of dry C.P. NaCl in 500 cc. 1 cc. equals 20 mgm. of NaCl.

Solution B:

100 cc. of solution A, diluted to 1000 cc. 1 cc. equals 2 mgm. of NaCl.

Solution C:

0.500 gram of NaCl in 1000 cc. 1 cc. equals 0.500 mgm. of NaCl.

II. Standard silver nitrate.

Make an approximately 0.02 N solution $AgNO_3$. (3.39 grams $AgNO_3$ per 1000 cc.

Standardize against standard NaCl solution B as follows: To 2 cc. of solution B. add 3 cc. of alcohol-ether and 1 drop of the indicator. Titrate with the approximate 0.02 N $AgNO_3$ to first definite pink color.

III. Alcohol-ether.

3:1 solution of 95 per cent ethyl alcohol and U. S. P. ether.

IV. Dichlorofluorescein indicator—Eastman Kodak Company (No. 373). 0.05 per cent in 70 per cent alcohol.

Procedure:

A. Blood serum:

1. To 5 cc. alcohol-ether in a 15 cc. centrifuge tube, add 0.2 cc. of serum with shaking.
2. Stopper tube and shake thoroughly.
3. Centrifuge at high speed for five minutes.
4. Add 2 drops of indicator.
5. Titrate in same tube with standard $AgNO_3$ until first definite pink color appears. The $AgNO_3$ must be standardized, as above with each chloride determination.

Calculation:

If 2 cc. standard NaCl Solution B require 3.4 cc. $AgNO_3$, and (2 cc. standard NaCl Solution B equals 4 mgm. NaCl), the 0.2 cc. serum requires 1.10

cc. $AgNO_3$, the proportion is 4 mgm. NaCl: 3.4 cc.:: x milligram: 1.10 cc. $3.4x = 4.40$. $x = 1.29$ mgm. NaCl in 0.2 cc. serum, therefore, 1 cc. serum contains 5×1.29 mgm. of NaCl or 6.45 mgm.; therefore 645 mgm. per cent NaCl in serum.

B. Cerebrospinal fluid:

0.2 cc. of spinal fluid are pipetted into test tube of 30 cc. capacity.
Wash sides of tube with 1 cc. of distilled water.
Add 3 cc. alcohol-ether mixture.
Add 2 drops indicator.
Titrate with $AgNO_3$ to first definite pink color.

Calculation same as for serum.

Comparison of values

	Wilson and Bell *mgm. per cent*	Volhard *mgm. per cent*	Adsorption *mgm. per cent*
Spinal fluid.....		731	735
Serum.....	575		563

Advantages claimed by the authors:

More accurate, rapid, and economical than other methods.
Fewer standard solutions required.
Complete analysis performed in one tube without transfer.

—Method suggested for trial by Dr. A. S. Giordano

SELENIUM AS A CATALYST IN DETERMINATION OF NON-PROTEIN NITROGEN IN BLOOD

FREDERICK REIS AND HARRY H. POWERS, J. Lab. and Clin. Med., **20:** 1204–1206. 1935

The digestion is carried out as with the Folin-Wu method. In place of charring, a rust-colored precipitate of reduced selenium appears. This dissolves as heating continues. If a slight brown color persists after ten minutes digestion, it may be disregarded; for it does not affect the accuracy.

Gum ghatti is used in this procedure to prevent turbidity.

Reagents required:

1. Standard ammonium sulphate containing 0.1 mgm. N per cubic centimeter 0.47165 gram ammonium sulphate in 1 liter distilled water.
2. Nessler's reagent:

Nessler's stock solution:

150 grams potassium iodide.
100 grams iodine.
140 to 150 grams mercury.
100 cc. water.
Add in order given.

Shake in Florence flask until color fades.
Cool under cold water.
Final product is greenish-yellow.
Make to 2 liters with distilled water.

Nessler's working solution:

1400 cc. of 10 per cent NaOH.
300 cc. Nessler's stock solution (formula given above).
300 cc. water.

3. Gum Ghatti solution:

Suspend 20 grams gum ghatti in a liter of distilled water over night.

4. Selenium-sulfuric acid:

Dissolve with heat 70 mgm. selenium in 90 cc. of sulfuric acid.
Cool and dilute to 200 cc. with distilled water.
It may also be prepared by adding 5 cc. of 2 per cent selenious acid to 200 cc. of 45 per cent sulphuric acid.

Determination:

Transfer 5 cc. of Folin-Wu filtrate to a Pyrex tube graduated at 35 cc. + 50 cc.

When ready to digest, add 1 cc. of well-shaken selenium sulphuric acid.

Add an anti-bump bead or tube.

Use a micro-burner having tube ¾ inch above flame, and digest at moderate speed.

If digested too rapidly all the organic matter is not oxidized, for it crawls upon the side of the tube and sticks there.

When SO_2 fumes appear, cover with a watch glass (mouth of the tube), and continue the digestion to completion.

Cool thoroughly and add approximately 25 cc. of distilled water.

Into another tube (35 cc. + 50 cc.) pipette:

1.5 cc. standard ammonium sulphate solution.
1. selenium-sulphuric acid.
25 cc. water.
Add 2 cc. gum ghatti solution both to the standard and to the unknown.
Mix.
Add 15 cc. Nessler's solution to each.
Dilute to 50 cc. mark with distilled water.
Mix and read.

The authors have found it best to Nesslerize and mix one tube at a time to avoid turbidity.

Calculation:

$$\frac{ST}{R} \times 30 = \text{milligram N per 100 cc. blood.}$$

Normal range = 25 to 35 mgm. per cent.

Advantage claimed by author is the elimination of the troublesome precipitate of silica caused by the action of phosphoric acid on the glass.

—Recommended for trial by Dr. A. S. Giordano.

BLOOD CALCIUM DETERMINATION USING STANDARD CALCIUM CHLORIDE SOLUTION

ISRAEL SCHWARTZ, J. Lab. and Clin. Med., **21**: 425–428. 1935

Caution:

Be sure all glassware used is clean and well rinsed with distilled water.

Principle:

The calcium in the serum is precipitated as calcium oxalate, and the precipitate is titrated with potassium permanganate in an acid medium.

Solutions:

1. 3 per cent ammonium oxalate.
2. 0.03 to 0.04 gram potassium permanganate dissolved in distilled water and made up to 100 cc. Keep in a dark bottle.
3. Standard calcium chloride solution. Dissolve 0.2498 gram of pure calcite ($CaCO_3$) Baker's in a 1:20 dilution of hydrochloric acid. Weigh the calcite into a 100 cc. beaker. Add the dilute HCl drop by drop, stirring after each addition. When the acid is added to the calcite, there is an evolution of carbon dioxide, which, *unless extreme care is taken*, will cause severe spattering and possible loss of calcium. When approximately 3 cc. of dilute HCl have been added to the calcite, add concentrated HCl one drop at a time and with thorough stirring, until solution is complete. Then carefully evaporate the solution almost to dryness on a steam bath. Beware of spattering. Take the residue up in approximately 2 cc. water and again evaporate almost to dryness. This process expels the last traces of HCl and is repeated four times. Dissolve the residue in distilled water and make up to 1 liter in a volumetric flask. One cubic centimeter of this standard $CaCl_2$ solution equals 0.1 mgm. Ca.

Procedure:

To 15 cc. centrifuge tube add:

2 cc. distilled water.

1 cc. of 3 per cent ammonium oxalate.

2 cc. of serum, drop by drop.

Mix very well.

Let stand 30 minutes, or longer. Allowing the precipitate to form in the ice box overnight is recommended.

Centrifuge 10 minutes, pour off the supernatant fluid, drain in the upright position, mouth downward on filter paper.

Wash precipitate three times with 2 per cent ammonium hydroxide, being sure that precipitate is stirred up each time so as to be well washed.

Stir the precipitate by using a rotary motion of the hand, rather than by using a stirring rod.

Dissolve precipitate by adding 2 cc. of approximately normal sulphuric acid and placing tubes in a 75°C. water bath.

Simultaneously employ 2 cc. of the standard calcium chloride solution and treat exactly the same as the serum.

Titrate the precipitate with the potassium permanganate solution using a clean stirring rod. Add potassium permanganate slowly and keep the solution warm.

Calculation:

C = milligram Ca in standard calcium chloride solution.

U = cubic centimeter of potassium permanganate used in titrating serum.

S = cubic centimeter potassium permanganate used in titrating the standard calcium chloride.

$$\frac{C \times U}{S} \times 50 \text{ mgm.} = \text{Ca per 100 cc. of serum.}$$

Multiply by 50 because 2 cc. of serum were used for the analysis, therefore 50 × 2 gives the milligrams of Ca in 100 cc. of serum.

Author's claims: Accuracy, dependability, ease of performance, simplification, lack of need for standardization of permanganate solution and permanency of calcium chloride solution.

—*Recommended for trial by Dr. A. S. Giordano.*